



Yin, Z., Cunningham, J., Vargas, K., Bengtson, S., Zhu, M., & Donoghue, P. (2017). Nuclei and nucleoli in embryo-like fossils from the Ediacaran Weng'an Biota. *Precambrian Research*, 301, 145-151. <https://doi.org/10.1016/j.precamres.2017.08.009>

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# Nuclei and nucleoli in embryo-like fossils from the Ediacaran Weng'an Biota



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## ARTICLE INFO

### Keywords:

Fossil embryo  
Doushantuo  
Animal  
Evolution  
Ediacaran

## ABSTRACT

The embryo-like microfossils from the Ediacaran Weng'an Biota (ca. 609 million years old) are among the oldest plausible claims of animals in the fossil record. Fossilization frequently extends beyond the cellular, to preserve subcellular structures including contentious Large Intracellular Structures (LISs) that have been alternately interpreted as eukaryote nuclei or organelles, degraded remains, or abiological structures. Here we present new data on the structure, morphology, and development of the LISs in these embryo-like fossils, based on Synchrotron Radiation X-ray Tomographic Microscopy (SRXTM) and quantitative computed tomographic analysis. All the lines of evidence, including consistency in the number, shape, position, and relative size (LIS-to-cytoplasm ratio) of the LISs, as well as their occurrence within preserved cytoplasm, support their interpretation as cell nuclei. Our results allow us to reject the view that nuclei cannot be preserved in early eukaryote fossils, offering new potential for interpreting the fossil record of early eukaryote evolution.

## 1. Introduction

As one of the oldest plausible claims of animals in the fossil record, the embryo-like microfossils from the Ediacaran Weng'an Biota (ca. 609 million year old (Zhou et al., 2017)) have long been expected to afford new insights into the developmental evolution of animal body plans (Chen et al., 2006, 2009; Xiao et al., 1998; Yin et al., 2016). However, the interpretation of these fossils has proven contentious because they preserve little more than simple geometric arrangements of cells that are not phylogenetically informative (Cunningham et al., 2017; Xiao et al., 2014). Though the biological interpretation of the fossils is debated, they are among the most remarkable instances of fossilization, not merely preserving component cells, but also intracellular structures (Hagadorn et al., 2006). These include small features interpreted as lipid vesicles or yolk granules (Hagadorn et al., 2006) and Large Intracellular Structures (LISs) whose interpretation is more controversial (Schiffbauer et al., 2012; Xiao et al., 2012, 2014). The LISs have a consistent size and location, sometimes occur paired in cells, positioned parallel to an anticipated plane of cell division (Hagadorn et al., 2006), and can be elongated or dumbbell-shaped, suggesting ongoing division (Hultgren et al., 2011). They were originally considered as nuclei, spindle bundles, or other organelles (Hagadorn et al., 2006) and most subsequent biological interpretations have focussed on a nucleus

interpretation (Chen et al., 2009; Hultgren et al., 2011, 2012; Schiffbauer et al., 2012; Xiao et al., 2012). This interpretation has been controversial, perhaps because details of cytokinesis have been invoked to exclude the affinity of fossils from crown-Metazoa, but also because of a prevailing notion that nuclei cannot be fossilized in early eukaryote microfossils (Francis et al., 1978; Knoll and Barghoorn, 1975; Pang et al., 2013; Xiao et al., 2014). Hence, we sought to test the established taphonomic models for the LISs and to reassess their origin not only to constrain affinities of these embryo-like fossils but also to better understand the early eukaryotic fossil record.

The principal arguments against the interpretation of the LISs as nuclei are, firstly, that they are incomparably large, larger than nuclei in extant eukaryotes, and larger than whole cells in putative later developmental stages of the same fossils – which would have been too small to contain nuclei with the same genetic material, not least since the LISs do not maintain a constant volume ratio with their host cells (Schiffbauer et al., 2012; Xiao et al., 2012, 2014). Secondly, Weng'an fossils exhibit many phases of mineralization, only the earliest of which is associated with the replication of biological structures (Cunningham et al., 2012, 2014; Schiffbauer et al., 2012). LISs are associated with late-stage void-filling diagenetic cements and, thus, even if the structures were formed at the sites of former nuclei or any other organelle, it has been argued that they will have been altered beyond interpretation by degradation and diagenetic

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<http://dx.doi.org/10.1016/j.precambres.2017.08.009>

Received 12 June 2017; Received in revised form 30 July 2017; Accepted 7 August 2017

Available online 08 August 2017

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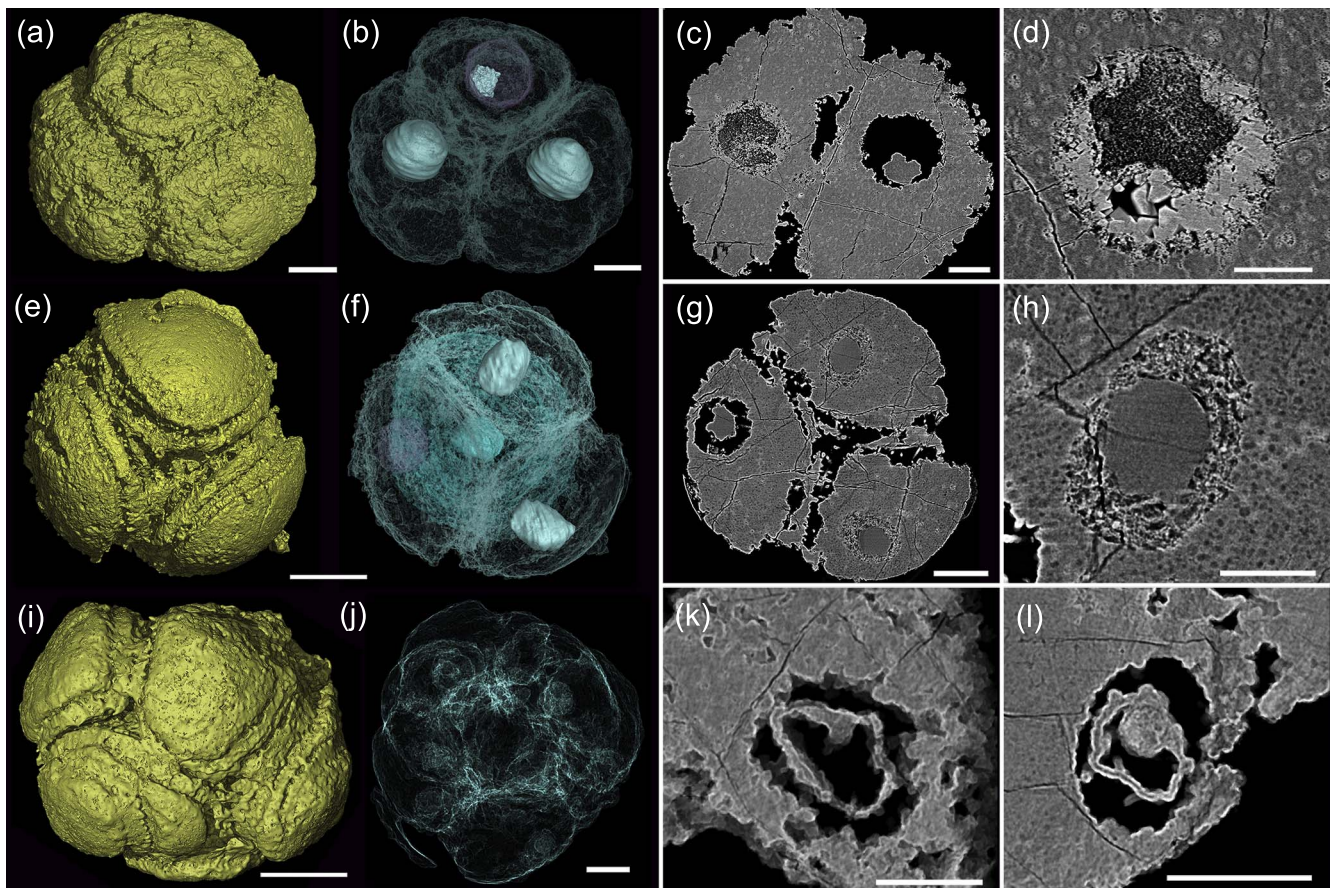


Fig. 1. Three specimens of *Spiralicellula*. (a) NIGPAS-166380, (b) Transparent mode, (c) Virtual section, (d) Close up view of a LIS. (e) NIGPAS-166381, (f) Transparent mode, (g) Virtual section, (h) Close up view of the ovoid LISs of (e). (i) NRM-PZ X 6774, (j) Transparent mode, (k, l) Slice slabs displaying details of two LISs in different cells. Note that the LISs in pink of (b) and (f) are hollow. Scale bar, 100  $\mu\text{m}$  for (a, b), 70  $\mu\text{m}$  for (c), 50  $\mu\text{m}$  for (d, h), 150  $\mu\text{m}$  for (e, f, i), 95  $\mu\text{m}$  for (g), 90  $\mu\text{m}$  for (j), 55  $\mu\text{m}$  for (k, l). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mineralization (Pang et al., 2013; Schiffbauer et al., 2012; Xiao et al., 2012, 2014). We attempted to test the competing interpretations of the LISs on the basis of new data on the structure and morphology of the LISs in the Weng'an embryo-like fossils *Tianzhushania* and *Spiralicellula*, obtained using Synchrotron Radiation X-ray Tomographic Microscopy and quantitative computed tomographic analysis. These data include specimens lacking post-decay void-filling mineralization, which allows us to further test alternative taphonomic models used to interpret the origin of LISs (Pang et al., 2013; Schiffbauer et al., 2012; Xiao et al., 2012, 2014) and reassess their origin.

## 2. Materials and methods

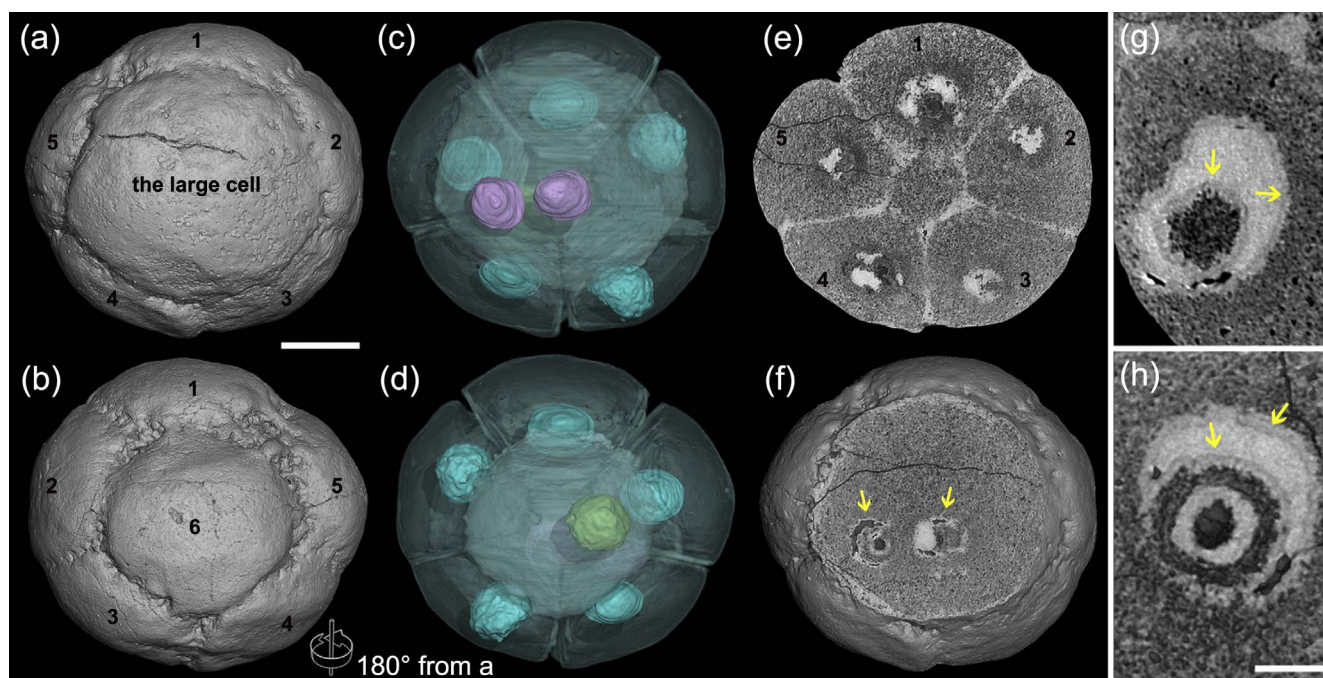
Figured specimens originate from the Upper Phosphorites of Ediacaran Doushantuo Formation in Datang, Nanbao and 54 quarries, Weng'an County, Guizhou Province, South China (Chen, 2004). Rock samples were dissolved in ca. 8–10% acetic acid and separated from the resulting residues by manual picking under a binocular microscope. The SRXTM analyses were carried out using the TOMCAT (X02DA) beamline at the Swiss Light Source and the ID19 beamline at the European Synchrotron Research Facility, using methods outlined previously (Donoghue et al., 2006; Tafforeau et al., 2006). The SRXTM data were visualized and measured using Avizo and VG Studio Max (2.2) software. Specimens are deposited in the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences (NIGPAS-166378 to NIGPAS-166381) and Swedish Museum of Natural History, Stockholm, Sweden (SMNH X 4403-4416; NRM-PZ X 6771 to NRM-PZ X 6775). Following best practise for digital morphology (Davies et al., 2017), our tomographic datasets and models are available from <http://dx.doi.org/10.5523/bris.2v3sw3xjkaum724prslziow4u0>. We follow Yin et al.

(2004) and Cunningham et al. (2017) in considering *Megasphaera* and *Parapandorina* as junior synonyms of *Tianzhushania*.

## 3. Results

Among hundreds of specimens of *Tianzhushania* and *Spiralicellula* characterised using SRXTM, tens exhibit LISs. The volume of LISs in 16 specimens of *Tianzhushania* from Datang Quarry range between 0.00003 and 0.00079  $\text{mm}^3$ , 2 specimens of *Tianzhushania* from 54 Quarry range between 0.00259 and 0.00877  $\text{mm}^3$  and 4 specimens of *Spiralicellula* range between 0.00021 and 0.00147  $\text{mm}^3$ . In most specimens, the LISs occur in the centre of each cell, but in some they are positioned eccentrically (Figs. 1–3). Their shape varies between specimens from approximately spherical or ovoid with a circular outline in section (Fig. 1c–h), to more irregular and kidney-shaped (Fig. 2g). There is usually only one such structure in each cell (Figs. 1 and 3), though there are exceptions. A seven-cell specimen of *Tianzhushania* from 54 Quarry has six small cells, each with a single LIS, and one large cell with two LISs (Fig. 2, arrows in f). The volume of the two LISs in this large cell (0.00259  $\text{mm}^3$  and 0.00313  $\text{mm}^3$ ) falls within the range of LISs in the other six small cells (0.00309  $\text{mm}^3$ –0.00435  $\text{mm}^3$ , mean = 0.00367  $\text{mm}^3$ ). The large cell containing two LISs has a volume of 0.124  $\text{mm}^3$ , which is approximately double the volume of the remaining small cells (0.057–0.082  $\text{mm}^3$ , mean = 0.068  $\text{mm}^3$ ). A six-cell specimen of *Tianzhushania* from Datang Quarry has two larger cells (0.0188  $\text{mm}^3$  and 0.0189  $\text{mm}^3$ ) that are each approximately twice the volume of the remaining four cells (0.0091  $\text{mm}^3$ –0.010  $\text{mm}^3$ , mean = 0.0096  $\text{mm}^3$ ). The volume of the LISs in the two larger cells (0.00039  $\text{mm}^3$  and 0.00053  $\text{mm}^3$ ) is also approximately double that of the LISs in the four smaller cells (0.00014  $\text{mm}^3$ –0.00023  $\text{mm}^3$ , mean = 0.00021  $\text{mm}^3$ ).



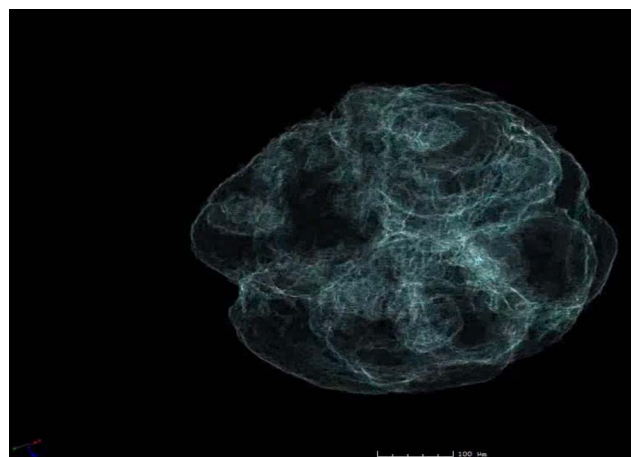


**Fig. 2.** A seven-cell specimen of *Tianzhushania* (NIGPAS-166378). (a) Bottom view, showing the largest cell surrounded by other five small cells. (b) Top view, showing six small cells. (c, d) Transparent modes of (a) and (b), respectively, showing eight LISs. Note that the largest cell contains two LISs (pink in (c)) and each small cell contains one LIS. (e) Digital section showing LISs in cells 1–5. (f) Digital section through the largest cell, showing two LISs (two arrows) inside the cell. (g, h) Details of LIS in cell 3 and 6, the yellow arrows indicating the low X-ray attenuation membrane-like structures in LISs. Scale bar, 250  $\mu\text{m}$  for (a)–(f) (in (a)); 75  $\mu\text{m}$  for (g, h) (in (h)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

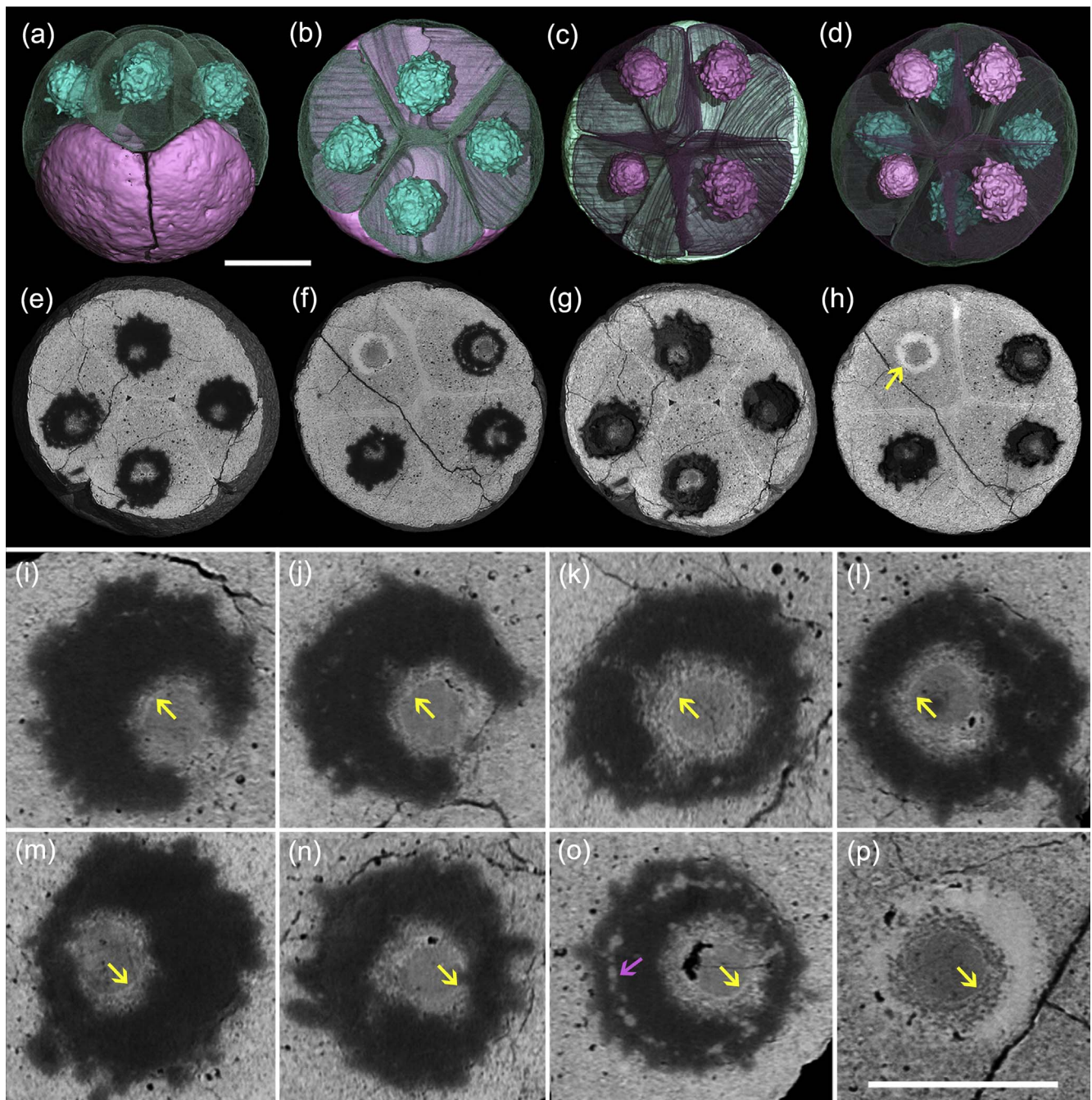
The relationship between cytoplasm and LIS volume from a large number of specimens exhibits some dispersion (Fig. 4), which is not surprising since the data encompass taxonomic, temporal, spatial and, doubtless, taphonomic variation. *Tianzhushania* specimens from Datang and 54 Quarry also differ considerably in terms of cell volume perhaps reflecting differences in biological affinity, or merely environmental, temporal, or preservational variation (diameters of 717 and 1125  $\mu\text{m}$  for the 54 Quarry specimens and an average of 579  $\mu\text{m}$  for the 16 specimens from Datang Quarry). However, there is a general trend for LISs to decrease in volume from 4- to 8- to 16-celled specimens (Fig. 4b). In the 6-cell *Tianzhushania* specimen, the four smaller cells plot in the same region as the cells of 8-celled specimens, while the two larger cells plot with cells from 4-celled specimens (Fig. 4b). For the Datang Quarry specimens, the mean ratio between LIS volume and cytoplasm volume (LIS/C ratio) is 0.021 for 4-celled *Tianzhushania* specimens, 0.022 for 8-celled specimens and 0.025 for 16-celled specimens (Fig. 4b). This suggests that there was a constant LIS/cell ratio, at least during early palintomy.

While the LISs are usually preserved with a coarse infill that is characteristic of post-decay diagenetic void-filling cement in Weng'an fossils (Fig. 1c, d, h) (Cunningham et al., 2012; Schiffbauer et al., 2012), our collections include specimens that lack this phase of mineralization such that the LISs are preserved as open voids (Fig. 1c, g, k, l). These contain much smaller irregular or kidney-shaped structures, which may be hollow, as seen in each cell of an eight-cell *Spirallicellula* specimen shown in Fig. 1i–l (Movie S1). In these specimens, the structures inside the voids are preserved in the same microcrystalline phase of mineralization as the rest of the specimen (Fig. 1c and g), with no visible inclusions. In other specimens, the LISs are preserved in a low X-ray attenuating phase of mineralization (Cunningham et al., 2012). This is seen in seven cells of an eight-cell specimen of *Tianzhushania* (Fig. 3), where each of these cells preserves a higher attenuating region in the centre of the LIS (Fig. 3i–o). The LISs in these cells are close to spherical, with irregular projections on the surface (Fig. 3a–d, i–o). In the centre of the remaining cell (Fig. 3h and p), a structure is preserved in the highly attenuating void-filling mineral phase that is more typical for

LISs, and has a lower attenuating region in the centre. This LIS is smaller than the very low attenuation structures in the other cells. Therefore, the irregular low attenuation projections in the other LISs likely extend beyond the original boundary of the structure. In some cases, a high attenuation rim is preserved and may reflect the original margin (Fig. 3o, red arrow on the left). Globular structures within the central regions of the LISs of such specimens are preserved in very fine crystals and have slightly lower attenuation than the mineralized cytoplasm. These are surrounded by thin rims with high X-ray attenuation (Fig. 3i–p, yellow arrows). There is sometimes evidence of void-filling textures between the membrane-like structure and the low attenuation region that surrounds it, but we have not observed these inside the membrane-like structure. In some specimens (Fig. 1d, h), small ovoid structures ca. 20  $\mu\text{m}$  in maximum dimension (previously interpreted as lipid vesicles or yolk granules (Hagadorn et al., 2006)) are preserved in the cytoplasm, but not within the LISs.



**Movie S1.**



**Fig. 3.** An eight-cell specimen of *Tianzhushania* (NIGPAS-166379). (a) Lateral view with the top four cells in transparent mode. (b) Top view. (c) Bottom view with the bottom four cells in transparent mode. (d) The same view as (c), with all the eight cells in transparent mode, showing the spatial relations of the cells and LISs in cells. (e, f) Three-dimensional sections showing top (e) or bottom (f) four LISs. (g, h) The same sections as (e) and (f), respectively, with dark materials of LISs removed, showing three dimensional details of the spherical structures within the 7 LISs excepting the one marked by yellow arrow in (h). (i–p) Details of eight LISs, note each LISs contains one membrane-enclosed spherical structure (yellow arrows). Scale bar, 300  $\mu\text{m}$  for (a–e) (in a); 150  $\mu\text{m}$  for (i–p) (in p). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

#### 4. Discussion

It has been argued that the LISs within the Weng'an embryo-like fossils cannot be molds of nuclei because they are preserved as post-decay diagenetic, void-filling, botryoidal cements, rather than nanocrystals nucleated during early diagenesis (Xiao et al., 2012). Though the LISs are usually infilled with later diagenetic void-filling cement, it does not follow that these voids themselves have an abiological origin, or are merely a consequence of degradation of an original biological structure. Their endogenous biological origin is evidenced by our material in which the globular bodies within the LISs are defined by the

low attenuation, microcrystalline mineral phase that preserves the rest of the cell (Fig. 1a–h), and is characteristic of early mineralization associated with the replication of biological structure (Cunningham et al., 2012). The approximate consistency of the size, shape, and position of the LISs, along with the moldic preservation of their morphology in association with the early mineralization of the cytoplasm, makes a biological interpretation compelling. Taphonomic interpretation of the embryo-like fossils must accommodate *prima facie* evidence that the LISs are preserved as voids, maintained by a biological structure that was not mineralized while the rest of the cell was mineralized. The coarsely crystalline cement characteristic of later void-filling



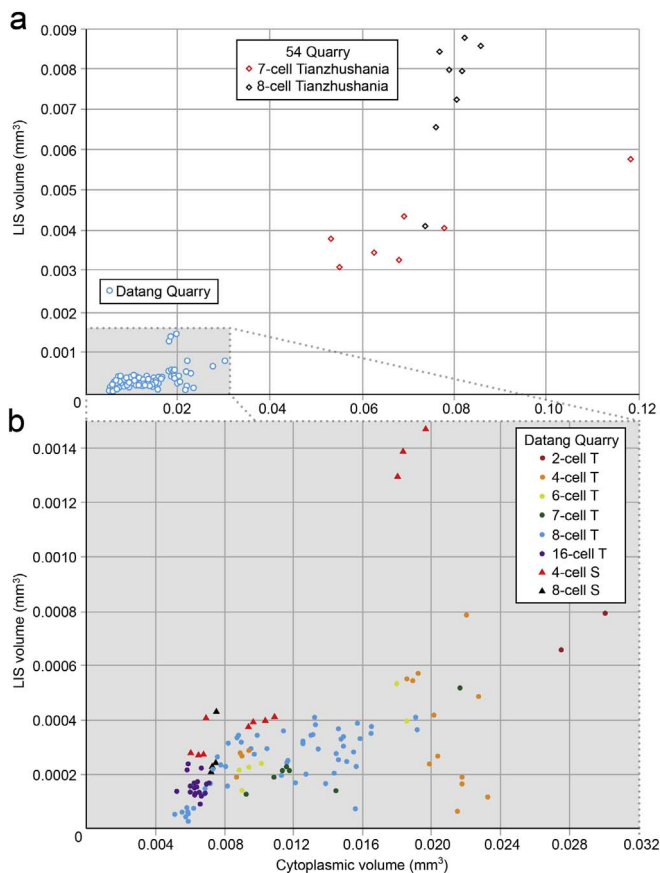


Fig. 4. Dimensions of cells and LISs. Numbers in the key refer to number of cells in each specimen. S = *Spirallicellula*, T = *Tianzhushania*. (b) The close up view of the lower left part of (a).

mineralization occurs in association with the LISs in most instances because they were preserved only as molds after decay of the constituent organics. The LISs cannot be interpreted as the shrunken remains of cytoplasm because of their consistent size, shape and position within cells, the fact that they contain consistent structures within them, and also because the cytoplasm itself is preserved through mineral replication.

Hagadorn et al. (2006) originally considered fossilized nuclei, spindle bundles, or other organelles as viable interpretations of the LISs. Other possibilities include mitochondria, vacuoles, nucleoli, chloroplasts, symbionts, or multi-membrane-bound organelles as in algal symbionts. However, the regularity of number, size, position and volumetric relationships, is compatible only with the nucleus interpretation (Huldtgren et al., 2011). This interpretation was subsequently rejected on the grounds that the LISs are unfeasibly large, and the cells of later developmental stages are smaller, too small to contain the same amount of genetic material (Xiao et al., 2012). However, given cell size, the nuclei are not unfeasibly large, or even unusually large for palintomically dividing cells (Huldtgren et al., 2012), and analyses have demonstrated an isometric relationship between cell and nucleus volume in living eukaryotes, like the one that we observe (Cavalier-Smith, 2005; Conklin, 1912; Goehring and Hyman, 2012; Hara and Kimura, 2009, 2011; Hara and Merten, 2015; Jorgensen et al., 2007; Neumann and Nurse, 2007; Tsihklaki and FitzHarris, 2016; Wilson, 1925). Furthermore, in living eukaryote systems, within a few rounds of palintomy, cell volume does indeed diminish to less than the original volume of the nucleus (Tsihklaki and FitzHarris, 2016). All the while, genome size is maintained through closer packing.

Accepting the nucleus interpretation, we turn to the interpretation of the intra-nuclear body that is preserved consistently, in the majority

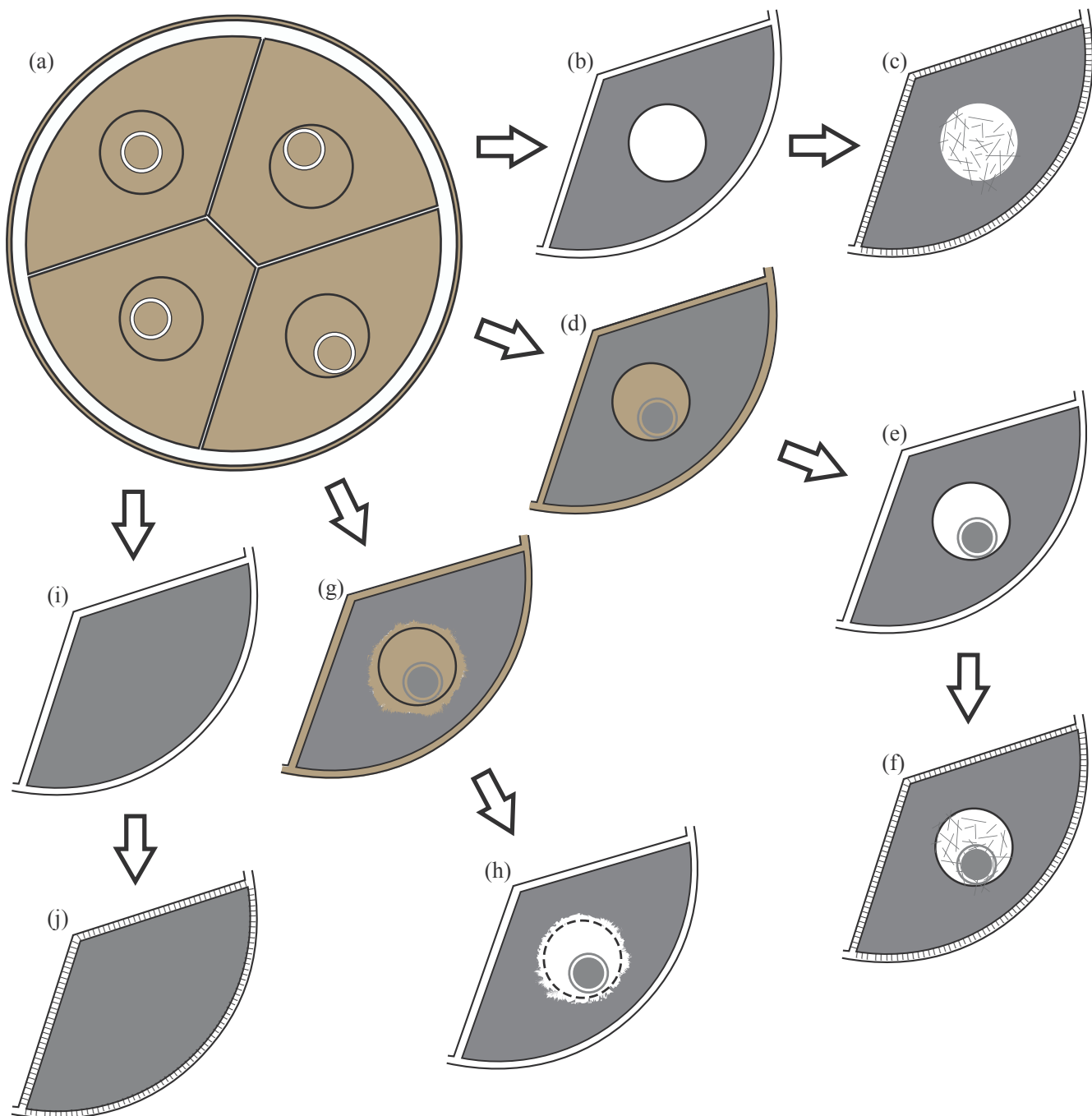
of specimens that preserve nuclei, regardless of whether the nucleus is preserved as an open void or infilled with later diagenetic cement. These structures, which occur in variable states of preservation and, therefore, regularity, are invariably preserved eccentrically, attached directly or indirectly to the margin of the nucleus void space. They have been referred to as nucleolus-like on account of their shape, position and consistent size (Chen et al., 2009; Huldtgren et al., 2011) but have conservatively been interpreted as likely artefacts, such as collapsed decayed remains of the nucleus (Cunningham et al., 2012; Huldtgren et al., 2011). However, among our specimens, these structures are sometimes preserved with a distinct membrane that is circular in cross section (Figs. 2g, 2h and 3i–p). This suggests that these structures are not shrunken nucleoplasm, or any other decayed cellular remains but, rather, distinct sub-nuclear biological structures. Among possible intranuclear structures, a nucleolus is the most viable interpretation, to which these structures compare favourably in terms of their relative size, shape and position within the nucleus.

We present a taphonomic model that rationalises the variation in cell, nucleus, and nucleolus preservation that we encountered in our dataset (Fig. 5). Confirmation of the preservation of nuclei and perhaps nucleoli in the Weng'an embryo-like fossils is helpful to discriminate among phylogenetic interpretations proposed previously. While our data do not add or detract from the interpretation of the fossils as non-metazoan holozoans (Huldtgren et al., 2011), they do nullify putative objections rooted in invalid preservational models that have sought to explain the preservation of these features (Xiao et al., 2012). Furthermore, confirmation of preserved remains of nuclei in the Weng'an embryo-like fossils has broader relevance in evidencing the possibility that nuclei can be fossilized. This is significant since it has been argued that nuclei cannot be fossilized in early eukaryote microfossils. This stems principally from debate over the bacterial versus eukaryote grade interpretation of cellular fossils from deposits such as the Gunflint (Awramik and Barghoorn, 1977) and Bitter Springs (Knoll and Barghoorn, 1975; Schopf, 1968) cherts. Taphonomy experiments have demonstrated that cytoplasmic remains in bacterial grade cells can shrink and condense to resemble a decayed nucleus (Francis et al., 1978; Knoll and Barghoorn, 1975). Thus, the fossilized intracellular structures have, understandably, been interpreted as decayed cytoplasm before. However, it should not be taken as evidence that nuclei cannot be preserved in the early fossil record (e.g. Pang et al., 2013). As we have shown with examples from the Weng'an deposit, this interpretation of the LISs as decayed cytoplasm can be rejected on evidence of the consistency of relative size, shape, and position of these structures and the preservation of unshrunken cytoplasm, as well as the consistent palintomic relationship to the surrounding cell and the preservation of differentiated structures in the putative nucleoli.

Further support from other deposits may be garnered from the report of preserved nuclei in an exceptionally permineralized Jurassic fern (Bomfleur et al., 2014), the veracity of which can be established on much the same grounds. Thus, the recognition of fossilized remains of nuclei in the Weng'an Biota opens a vista for the identification of fossils whose akaryote/eukaryote affinity is more contentious, helping to divine the roots of eukaryote-grade organisms in Earth History.

## 5. Conclusions

The consistent size, shape and position of the large intracellular structures within embryo-like fossils *Tianzhushania* and *Spirallicellula* and their occurrence within mineralized cytoplasm preclude their interpretation as artefacts resulting from taphonomic or diagenetic processes. Nuclei are the only intracellular structure that can account for the observed regularity in number, size, position, volumetric relationships of the LISs and the evidence for division in concert with the host cell. These findings confirm that the fossils are the remains of eukaryotes and not of bacteria as previously suggested (Bailey et al., 2007). The identification of nuclei within the Weng'an Biota, along



**Fig. 5.** Taphonomic pathways of Weng'an cells, nuclei, and nucleoli. (a) Living embryoid showing four cells containing nuclei which contain nucleoli. The brown colour indicates cytoplasm and nucleoplasm. (b, c) Pathway in which the cytoplasm is mineralized (dark grey) before nucleus later decays leaving a void (white) filled by diagenetic cement (slashes within the white void). (d–f) Pathway in which cytoplasm and nucleoli are mineralized (dark grey) before nucleus later decays leaving a void (white) filled by diagenetic cement (slashes within the white void) (refer to Figs. 2e, 3p). (g, h) Pathway in which cytoplasm is incompletely mineralized because of initial decay (dark grey indicating mineralized cytoplasm), nucleolus and nuclear envelope are mineralized along with amorphous decay products, leaving an otherwise unmineralized nucleus (white colour). The dashed black line in (h) represents incompletely mineralized nuclear envelope (refer to Fig. 3i–o). (i, j) Routine pathway in which the cell is mineralized as a homogenous structure because of initial decay of the nucleus and cytoplasm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with plausible reports from Phanerozoic deposits (Bomfleur et al., 2012, 2014; Matzke-Karasz et al., 2014; Ozerov et al., 2006), indicates that nuclei can be preserved in the fossil record, contrary to general expectation. Their preservation as external molds, in some instances in association with probable nucleoli, suggests that the nucleus was less susceptible to mineral replication than the surrounding cytoplasm. Our reassessment of the origin of these LISs in the Weng'an embryo-like fossils has potentially important implications for understanding Precambrian microfossils, where a record of nuclei or other organelles

could enable identification of early eukaryotes and help to constrain the timing and nature of eukaryotic evolution. Reports of preserved organelles in Precambrian fossils have generally lacked sufficient support and have consequently been discounted. However, the Weng'an embryoids suggest that revisiting these reports might be profitable.

#### Acknowledgements

We are grateful to Federica Marone (Swiss Light Source, Paul

Scherrer Institute) and Paul Tafforeau (European Synchrotron Radiation Facility) for assistance at their respective beamlines. This work is financed by Royal Society Newton Advanced Fellowship, the Ministry of Science and Technology of China (2013CB835000), the Chinese Academy of Sciences (XDB18030304), the National Natural Science Foundation of China (41672013), the Natural Environment Research Council (NE/J018325/1; NE/P013678/1), the Swedish Research Council (2013-4290), the Danish National Research Foundation (DNRF53), and the Science Without Borders Program (CNPq/Brazil).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.precamres.2017.08.009>. Tomographic data are available from <http://dx.doi.org/10.5523/bris.2v3sw3xjkaum724prs1ziow4u0>.

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